

A macroreticular resin is used as both an adsorbent for the analyte and the derivatizing reagent and also as a catalyst for the derivatization reaction. Subsequent serial elution of the absorbed compounds leads to simple separation of the derivatized analyte from the starting materials. Further, the resin impregnated with derivatizing reagent can be used to combine the extraction and derivatization of the analyte.

At column 7, lines 33-42, Rosenfeld indicates that in the case of prostaglandins double derivatization (esterification and oximation) is required for adequate analysis by gas chromatography with electron capture detection. Rosenfeld found that sequential derivatization is possible by employing his methods while the analyte is adsorbed on a single solid resin. In this way automation of an analytical technique was realized.

The present invention of Claim 1 is directed to a method for enhancing the confidence in detecting the presence of an analyte in a sample suspected of containing the analyte. In the method, a combination of at least two predetermined derivatives of the analyte is subjected to chromatographic separation. The predetermined derivatives exhibit different retention times as a result of the chromatographic separation. Then, the retention times of the derivatives are determined. The retention times are related to the presence of the analyte in the sample. The predetermined derivatives are selected on the basis of their ability to yield different retention times in the chromatographic separation of the derivatives and/or in their ability to yield different and/or additional detector responses. The number and intensities of the responses are related to the presence and/or amount of the analyte in the sample. Confirmation that the analyte is the analyte in question is then made based on the predicted or known retention times of the predetermined derivatives, separated or not, as well as the expected ratio of the intensities of the responses. The appearance of each of the derivatives at the expected times with the expected ratios indicates the presence of the analyte in the sample.

The Examiner asserts that Rosenfeld teaches a method of analyzing prostaglandin E2 (PGE2) using PFBr and PFBHOX derivatizing agents *in situ* on a column and then subjecting the samples to gas chromatography and electron capture where the trace is shown in Fig. 8. The Examiner argues that two peaks are labeled PGE2, indicating that two derivatives are present. The Examiner then continues by asserting that the x-axis on the chromatographic trace is time because

of the labeling of "start" and "stop" at the beginning and the end of the trace. The Examiner further refers to Example 16 in support of the above position.

Applicant submits that the above argument misconstrues the teaching of Rosenfeld. PGE2 has both carboxylic acid and carbonyl groups or functionalities, which, teaches Rosenfeld, both need to be derivatized in order to subject the PGE2 to gas chromatography with electron capture detection. Presumably, this is so because derivatization at both the carboxylic acid and carbonyl groups is necessary to increase intensity of signal so that analysis by gas chromatography and electron capture will yield a meaningful result. As indicated by Rosenfeld at column 7, lines 33-38, the method of Rosenfeld may be employed to achieve derivatization of both of the above functionalities to form a derivatized product, i.e., a single product in which both the carboxylic acid and carbonyl groups are derivatized. This is supported by the comments of Rosenfeld, for example, at column 23, line 3, where Rosenfeld refers to reaction at two positions of PGE2, and column 20, lines 25-26, where Rosenfeld refers to the isolation of derivatized analyte.

The Examiner refers to Figure 8. First of all, Applicant does not dispute that Figure 8 depicts retention times. Rosenfeld refers to retention times at column 21, lines 13-16. At the above passage, Rosenfeld indicates that for PGE2 the retention time of the minor isomer was 16.73 minutes and of the major isomer was 17.53 minutes and the retention time of the external control was 12.94 minutes. Rosenfeld does not teach that two separate derivatives are being employed. Rosenfeld is clearly referring to the derivatized products of two isomers of PGE2, where each product has both the carboxylic acid and carbonyl groups derivatized by ester and oxime formation, respectively. The two isomers of PGE2 represent two distinct analytes. There is only a single derivative for each of these analytes, which are represented in Figure 8 by two distinct lines.

As Applicant indicated in the Specification, the formation of a single derivative of an organic compound as a step during its analysis as well as derivatizing reagents for forming such a derivative are known. For example, where the analysis involves highly sensitive techniques, the presence of certain specific groups in the molecule such as, for example, chromophores, fluorophores or electrophores is required. Many organic compounds do not possess such groups and must be converted to a suitable derivative as a preliminary step of the analysis. In some cases it is also necessary to convert an unstable compound to a stable derivative. Applicant

continued by indicating that, in the present invention, two or more predetermined derivatives for each analyte, rather than a single derivative for each analyte, are used thereby creating multiple peaks of lower intensity than any single derivatization alone. This approach runs counter to the general thinking in the art that purified components are necessary in order to obtain a single peak and high intensity thereby maximizing the possibility of achieving the detection and correct identification of the analyte.

In the disclosure of Rosenfeld embodied in Figure 8, there are two different analytes, i.e., two isomers of PGE2, which have been derivatized with PFBr and PFBHOX to form a single derivatized product for each analyte. There is no disclosure or suggestion of the presently claimed invention. The invention of Rosenfeld relates to the use of a resin to extract analyte and to form derivatized analyte. Rosenfeld's disclosure does not go beyond teaching analysis using one derivative per analyte. As Rosenfeld indicates at column 7, lines 50-51, his invention can provide the necessary automated extraction and derivatization for a system for routine clinical analysis.

The Examiner also refers to Figures 4A and 4B and asserts that Rosenfeld additionally teaches separation of derivatives of THC formed metabolically and further derivatized *in situ*. Example 18 of Rosenfeld provides the disclosure surrounding Figures 4A and 4B. Again, Rosenfeld discloses only a single derivative for each analyte. Figures 4A and 4B depict three different analytes, namely,  $\Delta^9$ -THC, cannabinol and  $\Delta^9$ -11-OH-THC. Only single derivatives are shown for each of these analytes, namely,  $\Delta^9$ -THC-(PFB), cannabinol-(PFB) and  $\Delta^9$ -11-OH-THC-(PFB)-(TMS), respectively. Figure 4A depicts the known approach to the synthesis of the derivative of the respective analytes while Figure 4B depicts the synthesis of the derivative of the respective analytes according to the invention of Rosenfeld (column 18, lines 19-22). Accordingly, Figures 4A and 4B do not disclose or suggest the methods of Claims 1-6.

#### Rejection under 35 U.S.C. §103

Claims 1-22 were rejected under paragraph (a) of the above code section as being unpatentable over Rosenfeld (U.S. Patent No. 4,990,458). Applicant submits that the comments made above apply equally to this ground of rejection as set forth

in paragraphs 6-8 of the Office Action. There is no suggestion in Rosenfeld to make more than one derivative of an analyte and subject a combination of the derivatives to chromatographic separation and then to relate the retention times to the presence of the analyte in the sample.

There is no teaching or suggestion in Rosenfeld of an embodiment of the present invention comprising subjecting a combination comprising at least two predetermined derivatives of an analyte to chromatographic separation to separate the derivatives, subjecting the derivatives to ionization to form ions as they exit from the chromatograph, detecting a response from each of the ions, determining retention times of the ions and the ratios of the intensities and relating the retention times and the ratios to the presence and/or amount of the analyte in the sample. It is not Applicant's position that Rosenfeld does not teach retention times. It is Applicant's position that Rosenfeld does not teach making at least two predetermined derivatives of an analyte and subjecting the derivatives to the aforementioned steps.

There is no teaching or suggestion in Rosenfeld of an embodiment of the present invention with the steps of combining a sample with at least two predetermined derivatizing agents and then subjecting the combination to conditions under which derivatives of the analyte are formed, subjecting the derivatives to gas chromatographic separation to separate the derivatives, subjecting the separated derivatives to negative ion chemical ionization to form negative ions of the derivatives, subjecting the ions to mass analysis and detecting a response from the ions, determining the retention times of the ions and the ratios of the intensities of the responses and relating the retention times and the ratios to the presence and/or amount of the drug in the sample.

#### Prior Art Made of Record

Applicant acknowledges the Examiner's at least implicit finding that Apffel, et al. (U.S. Patent No. 4,784,962) (Apffel), either individually or in combination with Rosenfeld, does not disclose or suggest the present invention. Apffel discloses preparing an OPA derivative of each primary amino acid in a mixture of amino acids and an FMOC derivative of each secondary amino acid in a mixture of amino acids. There is no disclosure or suggestion in Apffel to make more than one derivative of each amino acid analyte.

Formal Drawings

Applicant acknowledges the Examiner's indication that the proposed drawing correction filed on February 9, 2001, was approved. Accompanying this response are formal drawings, which include the approved amended matter.

CONCLUSION

Claims 1-22 satisfy the requirements of 35 U.S.C. 102 and 103. Allowance of the above-identified patent application, it is submitted, is in order.

Respectfully submitted,



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